Adhesion of *Candida albicans* to Endothelial Cells under Physiological Conditions of Flow\(^\ddagger\)†

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*Candida albicans* is a commensal organism that under certain circumstances can become pathogenic. During systemic infection *C. albicans* is disseminated via the circulation to distant organs, where it causes multiple organ failure. Despite the severity of systemic *C. albicans* infection, little is known about the mechanisms involved in the adhesion of this organism to the endothelium lining blood vessels. Previous studies have used static assays to examine adhesion. However, these do not realistically model blood vessels, where circulating *C. albicans* cells must adhere to the endothelium under conditions of flow and shear stress. Furthermore, there is conflicting evidence concerning the role played by yeast, pseudohyphal, and hyphal forms of *C. albicans* in adhesion to endothelium. To test the hypothesis that there may be differences in the abilities of these three morphogenic forms of *C. albicans* to adhere to endothelium under conditions of flow, we developed an in vitro flow adhesion assay. We found that all three forms of *C. albicans* rapidly bind to confluent endothelial cells under conditions of flow. Maximum adhesion was found at low shear stress levels similar to that found in postcapillary venules. Moreover, yeast forms bound in significantly greater numbers than did pseudohyphal and hyphal forms, respectively, contrasting with previous findings from static assays. These findings are consistent with recent in vivo data suggesting that yeast forms may be capable of adhering to the endothelium and migrating into the tissues before undergoing morphogenic change to cause tissue damage.

*Candida albicans* is a normal commensal organism of the oral cavity, gastrointestinal tract, and vagina. Usually the organism colonizes these mucosal surfaces without causing infection. However, under certain circumstances it is capable of becoming pathogenic. Systemic candidiasis is a serious infection with a high mortality rate and morbidity in those who survive (26). After infections caused by methicillin (meticillin)-resistant *Staphylococcus aureus*, it is probably the second most common cause of death from hospital-acquired infections and involves the hematogenous spread of *C. albicans* to multiple organs (11, 27). Mucocutaneous candidal infections seldom lead to systemic candidiasis. Even fungal colonization of the lungs and gastrointestinal tract does not always lead to systemic infection but may occur when surface integrity and host defenses are impaired. Systemic candidiasis appears to occur most often when direct access of fungi is facilitated by major gastrointestinal or cardiac surgery, organ transplantation, or the use of indwelling intravascular catheters and devices (13).

During systemic dissemination, circulating *C. albicans* must first adhere to the endothelial cells lining blood vessels before transmigrating across the vessel wall and entering the tissues. Despite the morbidity caused by systemic candidiasis, little is known about the mechanisms involved in the adhesion of *C. albicans* to endothelial cells or their subsequent transmigration. Currently, there are two different theories as to how *C. albicans* adheres to the endothelium and enters the tissues (reviewed in reference 10). The first theory proposes that *C. albicans* must undergo morphogenic conversion from yeast to hyphal forms within the circulation to be able to invade the tissues (6, 19, 21). However, more-recent data indicate that morphogenic change may not be necessary, suggesting that yeast cells may be able to adhere to the endothelium and transmigrate into the tissues before undergoing morphogenic change (3, 4, 23, 24).

Studies investigating candidal adhesion to the endothelium have mainly used static in vitro assays where *C. albicans* is left in prolonged contact with cultured monolayers of endothelial cells. This is very different from the fleeting interactions that *C. albicans* has with endothelial cells under conditions of shear stress and flow that occur in blood vessels in vivo. Numerous studies with leukocytes, tumor cells, and microorganisms have shown that static assays do not replicate the dynamic interactions that occur with endothelium under conditions of flow and are poor at elucidating the contribution of specific adhesion molecules (8, 14). However, to date no studies have investigated the adhesion of the three different morphogenic forms of *C. albicans* to endothelial cells under conditions of flow.

To test the hypothesis that there may be differences in the abilities of yeast, pseudohyphal, and hyphal forms of *C. albicans* to adhere to the endothelium under conditions of flow, we developed an in vitro flow adhesion assay that mimics the conditions found within blood vessels. Here we show for the first time that adhesion of all three forms of *C. albicans* to
the endothelium under conditions of flow is rapid. In addition, yeast forms adhere with greater numbers than do pseudohy- 

al or hyphal forms, suggesting that yeast forms may be the most predominant form adhering to endothelial cells during systemic infection.

MATERIALS AND METHODS

C. albicans strains and culture conditions. The strains of C. albicans used in this study were the wild-type CAF2-1 strain (7) and the genetically modified tet-NRG1 strain SSY50-B, in which yeast-to-hyphal conversion is under the control of a tetracycline-regulatable promoter upstream of the NRG-1 gene (23).

For use in experiments, the organisms were grown overnight at 25°C in yeast extract-peptone-dextrose (YPD) broth (Oxoid, Hampshire, United Kingdom) with shaking at 100 rpm. Overnight cultures of CAF2-1 were then diluted (1:20) into three different culture broths to allow morphogenic conversion and incubated on a shaker. To ensure that CAF2-1 remained in yeast form, Candida cells were grown in YPD broth at pH 6 at 25°C; for conversion to pseudohyphae, CAF2-1 cells were grown in YPD broth at pH 7.5 at 35°C; for conversion to hyphae, CAF2-1 cells were grown in YPD broth with 2% fetal calf serum at pH 7 at 37°C (25). Hyphae and pseudohyphae were cultured for 1 h, giving rise to short germ tubes, and yeast cells were cultured for 3 h to ensure that the correct morphogenic form had been reached. Microscopy confirmed that at this time point the organisms remained as separate cells with no evidence of significant clumping. The 1-h time point was chosen for hyphae and pseudohyphae for three reasons. First, longer culture periods gave rise to longer germ tubes that lead to clumping and blockage within the flow assay. Second, hyphae with longer germ tubes would not continue to circulate in vivo, as they would be filtered out by the reticuloendothelial system. Lastly, previous static assays used hyphae cultured for 60 min, and so using this time point allowed us to compare our data with those published previously (28).

For the SSY50-B strain, overnight cultures were diluted (1:20) into YPD and cultured with or without 20 μg/ml doxycycline hyclate (Sigma, Poole, United Kingdom) at 37°C for 2 h to generate hyphal or yeast forms, respectively (23). Again microscopy confirmed no evidence of significant clumping. After culture in the appropriate media, the different strains and morphogenic forms of C. albicans were centrifuged at 300 × g for 5 min. Cells were then washed and resuspended in HEPES-buffered Hanks’ balanced salt solution (HBSS; Sigma, Poole, United Kingdom) at 1 × 10^6 cells/ml, vortexed to minimize clumping, and immediately used in flow experiments.

C. albicans cells were characterized as yeast, pseudohyphae, or hyphae by using a number of different methods including cell shape, extension width, and the presence of a constriction at the base of the mother cell. C. albicans cells were characterized as pseudohyphae when the extension was greater than 2 μm wide and characterized as hyphae when the extension was less than 2 μm wide. C. albicans cells were also characterized as pseudohyphae when there was a constriction at the base of the mother cell, which is indicative of pseudohyphae (Fig. 1) (25).

Endothelial cells. The immortalized human microvascular endothelial cell line HMEC-1 (1) (provided by F. J. Candia, Centers for Disease Control and Prevention, Atlanta, GA) was cultured in MCDB-131 (Gibco, Invitrogen, Paisley, United Kingdom) supplemented with 10% (vol/vol) fetal calf serum (BioSera Ltd., Ringmer, United Kingdom), 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 ng/ml epidermal growth factor, and 1 μg/ml hydrocortisone (Sigma, Poole, United Kingdom). Cells were incubated at 37°C in 5% CO₂ in tissue culture flasks and passaged by being incubated with HBSS containing no calcium or magnesium (Lonza, Cambridge, United Kingdom) followed by trypsin-EDTA (Sigma, Poole, United Kingdom). For use in flow experiments, HMEC-1 cells were cultured on sterilized glass slides in four-well plates (Nunc) for 72 h until confluent monolayers had formed. Any nonadherent cells were removed by being washed with HBSS prior to flow experiments.

Static assay. The static assay was conducted on glass slides coated with confluent HMEC-1 monolayers in four-well plates (Nunc) using modifications of the method described by Zhao et al. (28). Briefly, cells were rinsed with HBSS, after which 3 ml of Candida suspension (1 × 10^6 cells/ml in HBSS) was added to each well. After 15, 30, or 60 min of incubation at 37°C, the unbound organisms were aspirated and each well was washed four times with HBSS. Slides were fixed in 10% buffered formalin before adhesion was visualized and recorded using a Zeiss Axiovert 200 M inverted fluorescence microscope with an integrated high-resolution digital camera (AxioCam MRm; Zeiss) with AxioVision 4.6 software (Imaging Associates Limited, Bicester, United Kingdom). The total number of adherent cells/mm² was recorded. The number of adherent C. albicans cells represents the mean ± standard error of the mean (SEM) of at least 15 fields (field area = 0.15 mm²) from three independent experiments.

Flow adhesion assay. Glass slides coated with confluent HMEC-1 monolayers were mounted in a parallel plate flow chamber (GlycoTech, Rockville, MD), which was then placed on a 37°C stage in an environmental chamber also maintained at 37°C. C. albicans cells (yeast, pseudohyphal, or hyphal forms) at 1 × 10^6/ml (unless otherwise stated) were then perfused through the flow chamber and over the endothelial cell monolayers by using an automated syringe pump (Harvard Apparatus, Natick, MA). Adhesion of C. albicans to endothelial cells was visualized using a Zeiss Axiovert 200 M inverted fluorescence microscope. An integrated high-resolution digital camera (AxioCam MRm; Zeiss) with AxioVision 4.6 software was used to record the flow experiments (see Fig. 3). C. albicans suspensions were allowed to perfuse the flow chamber for 2 min before recording began. Results consisted of 15-min recordings of a random field of view using a 20× objective; the area of the analyzed field was 0.15 mm². Each experiment was repeated with three separate confluent endothelial cell slides at least three times. Cell motion analysis was performed using cell tracking software (Imaging Associates Limited, Bicester, United Kingdom). Images were acquired over 15 min into a video file at 2 frames/minute (unless otherwise stated), and the total number of adherent cells/mm² was recorded.

Flow rate calculations. To work out the required flow rate, the following equation was used:

\[ \tau_r = \frac{\mu Q}{w v} \]

where \( \tau_r \) is the wall shear stress (dynes/cm²), \( \mu \) is the apparent viscosity of the
TABLE 1. Shear stress values used in this study and their corresponding flow rates

<table>
<thead>
<tr>
<th>Shear stress (dynes/cm²)</th>
<th>Flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.2122</td>
</tr>
<tr>
<td>0.5</td>
<td>0.4244</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8489</td>
</tr>
<tr>
<td>1.5</td>
<td>1.2733</td>
</tr>
<tr>
<td>2.0</td>
<td>1.6978</td>
</tr>
<tr>
<td>3.0</td>
<td>2.5467</td>
</tr>
<tr>
<td>4.0</td>
<td>3.3956</td>
</tr>
</tbody>
</table>

medium (0.0076 P = water at 37°C), \( a \) is the height of the chamber (0.0254 cm), \( b \) is the width of the chamber (1 cm), and \( Q \) is the flow rate (ml/s). The flow rates that correspond to the levels of shear stress are shown in Table 1.

Statistical analysis. The differences between groups were analyzed by the Mann-Whitney U test, unpaired \( t \) test, or linear regression (GraphPad Prism version 5.01 for Windows; GraphPad Software, San Diego, CA). The results were considered statistically significant at a \( P \) value of \( < 0.05 \).

RESULTS

The effect of culture conditions on the morphogenetic form of \( C. \) albicans. To determine the composition of the three different morphological forms in the \( C. \) albicans preparations of CAF2-1, we examined samples from each culture condition prior to use in the flow assay using light microscopy and recorded the number of yeast, pseudohyphal, and hyphal forms (Fig. 1 and Table 2). Growth under culture conditions that promote either yeast, pseudohyphal, or hyphal forms of \( C. \) albicans resulted in the presence of 94%, 74%, and 92% of these morphological forms of wild-type CAF2-1, respectively. Hyphae grown for 1 hour have short germ tubes (~20 \( \mu \)m) and as a result are less likely to clump. In the absence of doxycycline, over 95% of cells of the SSY50-B strain of \( C. \) albicans were yeast forms, even under hypha-promoting conditions (23), whereas in the presence of doxycycline over 95% of cells were converted to the hyphal form.

Under static conditions, hyphal forms of \( C. \) albicans preferentially bind to endothelium. To investigate the adhesion of the different forms of \( C. \) albicans to endothelial cells under static conditions, yeast, pseudohyphal, and hyphal forms of CAF2-1 at \( 1 \times 10^6 \) cells/ml were incubated with confluent HMEC-1 monolayers and the number of adherent \( C. \) albicans cells was recorded (Fig. 2). After 15 or 30 min of contact, hyphae adhered in significantly greater numbers than did yeast forms (\( P = 0.0006 \)) but in lower numbers than did hyphal cells (\( P = 0.0136 \)). After 60 min pseudohyphal adhered less than did either yeast forms (\( P = 0.0099 \)) or hyphal (\( P = 0.0023 \))

The rate of flow and shear stress significantly affect adhesion of \( C. \) albicans to endothelial monolayers. To investigate the effect of flow rate and shear stress on adhesion of \( C. \) albicans to endothelial cells, yeast and hyphal forms of CAF2-1 at \( 1 \times 10^6 \) cells/ml were perfused over HMEC-1 monolayers at increasing levels of shear stress and the number of adherent \( C. \) albicans cells was recorded (Fig. 3). At a shear stress of 0.25 dynes/cm², sevenfold more yeast cells than the hyphal form bind to HMEC-1 monolayers (Fig. 4). As the shear stress increased, the number of adherent yeast cells declined in a shear stress-dependent manner (Fig. 4). However, at all shear stress levels tested, yeast forms of \( C. \) albicans were still significantly more adherent to endothelial cells than were hyphal forms (\( P = 0.05 \)). The overall proportion of \( C. \) albicans that adhere is small, and at 1.5 dynes/cm² only 1.1% of yeast cells and 0.2% of hyphal that pass through the flow chamber adhere to the endothelial cells. At shear stresses above 3 dynes/cm², the adherence of both forms of \( C. \) albicans to HMEC-1 cells was negligible and there was no significant difference in the adhesion of yeast and hyphae.

TABLE 2. Percentage of each morphological form of the CAF2-1 strain of \( C. \) albicans used in the flow assays

<table>
<thead>
<tr>
<th>CAF2-1 sample</th>
<th>Mean % ± SEM (n = 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>94.41 ± 1.00</td>
</tr>
<tr>
<td>Pseudohyphal</td>
<td>5.23 ± 1.01</td>
</tr>
<tr>
<td>Hyphal</td>
<td>0.36 ± 0.24</td>
</tr>
<tr>
<td>Yeast</td>
<td>3.55 ± 0.77</td>
</tr>
<tr>
<td>Pseudohyphal</td>
<td>74.46 ± 1.57</td>
</tr>
<tr>
<td>Hyphal</td>
<td>21.99 ± 1.46</td>
</tr>
<tr>
<td>Yeast</td>
<td>1.57 ± 0.81</td>
</tr>
<tr>
<td>Pseudohyphal</td>
<td>6.90 ± 1.64</td>
</tr>
<tr>
<td>Hyphal</td>
<td>91.52 ± 1.40</td>
</tr>
</tbody>
</table>

* More than 200 cells counted.
Yeast forms of C. albicans preferentially bind to endothelium under flow conditions. Preliminary experiments showed that yeast forms adhere significantly more to endothelial cells than do hyphal forms. To investigate this more vigorously, the influence of C. albicans morphogenic form on adhesion to endothelial cells was tested under two different shear stress rates. Firstly, $1 \times 10^6$ cells/ml of yeast, pseudohyphal, and hyphal forms of CAF2-1 were made to flow over confluent HMEC-1 monolayers for 15 min at different shear stresses (0.25, 1, 2, 3, and 4 dynes/cm²), and the number of organisms adherent to the endothelium per mm² was counted (median ± range, $n = 3$; * $P = 0.05$, Mann-Whitney U test).

To further rule out the effects of culture conditions on adhesion of C. albicans to endothelial cells under flow conditions, experiments were performed with Candida where changes in

FIG. 4. Effect of shear stress on the adhesion of C. albicans. Yeast and hyphal forms of C. albicans (CAF2-1) at $1 \times 10^6$ cells/ml were made to flow over confluent HMEC-1 monolayers for 15 min at different shear stresses (0.25, 1, 2, 3, and 4 dynes/cm²), and the number of organisms adherent to the endothelium per mm² was counted (median ± range, $n = 3$; * $P = 0.05$, Mann-Whitney U test).

FIG. 5. Yeast forms of C. albicans predominantly bind to endothelium under conditions of flow. Yeast, pseudohyphal, and hyphal forms of CAF2-1 (A and C) and SSY50-B with or without doxycycline (DOX) (B and D) at $1 \times 10^6$ cells/ml were made to flow over confluent HMEC-1 endothelial cells at 0.25 dynes/cm² (A and B) and 1.5 dynes/cm² (C and D) for 15 min, and the cumulative number of organisms adhering to the endothelium per mm² was counted after 5, 10, and 15 min (mean ± SEM, $n = 3$; linear regression).
morphology were induced by altering the temperature but maintaining the same pH (pH 7) and medium (YPD). This showed that yeast forms (cultured in YPD at 30°C and pH 7, 85% yeast) adhered more than did hyphae (cultured in YPD at 37°C and pH 7, 69% hyphae) (see Fig. S1 in the supplemental material). Furthermore, culturing CAF2-1 with different carbon sources (50 mM glucose or 500 mM galactose) did not alter the pattern of binding (see Fig. S2 in the supplemental material). These experiments show that culture conditions are not responsible for the greater adherence of yeast than of hyphae.

The effect of input concentration on the adhesion of C. albicans. To investigate the effect of input concentration on adhesion of C. albicans to endothelial cells, yeast and hyphal forms of CAF2-1 at increasing concentrations (0.1 \( \times \) 10^6, 0.5 \( \times \) 10^6, 1 \( \times \) 10^6, 2 \( \times \) 10^6, and 3 \( \times \) 10^6/ml) were perfused over confluent HMEC-1 monolayers at 1.5 dynes/cm^2 for 15 min, and the number of organisms adhering to the endothelium per mm^2 was counted (median \pm interquartile range, n = 6; *, \( P \leq 0.05; **, P \leq 0.001, \) Mann-Whitney U test).

endothelial cells (\( P = 0.0475, P = 0.0004, \) and \( P = 0.0054, \) respectively). This experiment shows that starting concentration does not influence the outcome of the results.

C. albicans cells adhere rapidly to endothelial cells under conditions of flow. It was notable that, when freely flowing through the flow chamber, pseudohyphal and hyphal candidal forms were oriented with their long axis parallel to the direction of flow with their mother cell leading and their hyphal/pseudohyphal extension trailing behind (Fig. 7). On adhering to the endothelial surface, all three forms of C. albicans abruptly arrested at the site of contact with no tumbling or rolling across the endothelial cell surface. This adhesion was extremely rapid, with organisms free flowing in one frame of the video and stationary in the next. With a frame rate of 6 frames s^{-1}, this indicates that firm adhesion occurs in less than 167 ms of contact with the endothelial surface. Once they were adherent, it was rare for organisms to become detached from the endothelium even at high levels of shear stress (up to 4 dynes/cm^2).

All three forms of C. albicans were noted to form heterotypic primary adhesions, i.e., direct adhesion with the endothelial cell surface, as well as homotypic secondary adhesions, i.e., adhering to C. albicans cells already bound to the endothelial cell surface. Thus, small aggregations of C. albicans would form around organisms that had undergone primary adhesion.

It was also notable that a marked number of hyphal and some pseudohyphal forms adhered to the endothelium by the tip of their hyphal processes (Fig. 7). As a result, hyphae and pseudohyphae adherent to endothelial cells (heterotypic primary adhesions) aligned with the direction of flow with their mother cell appearing unattached to the endothelium and being buffeted from side to side by the medium flowing past (Fig. 7, insets).

DISCUSSION

Dissecting the mechanisms involved in the adhesion and subsequent transmigration of Candida albicans in systemic candidiasis is complicated due to the conflicting evidence about the role of the different morphological forms and by the role of morphogenic change (10). Since adhesion to endothelium under conditions of flow is the critical first step in the passage of C. albicans from the circulation into the tissues, we developed
an in vitro flow adhesion assay to investigate the adhesion of yeast, pseudohyphal, and hyphal forms of \textit{C. albicans} to endothelial cells.

Previous studies using static assays show greater adherence of hyphae to endothelial cells (20). Our findings confirmed this and revealed that hyphal forms of \textit{C. albicans} adhered in significantly greater numbers than did yeast forms after 15 and 30 minutes. After 60 minutes there was no difference between adhesion of yeast forms and adhesion of hyphal forms to endothelial cells. However, this may be because the majority of yeast forms had become hyphal after 60 min of incubation under these culture conditions.

Static assays do not realistically model blood vessels in vivo, where circulating \textit{C. albicans} cells must adhere to the endothelium under conditions of shear stress. Our experiments showed that under conditions of flow, adherence of \textit{Candida} to confluent endothelial monolayers markedly decreased as the shear stress increased. These findings are consistent with previous studies involving leukocytes which also showed that increased shear stress leads to decreased leukocyte adhesion (15). \textit{C. albicans} adhered to the endothelium in greatest numbers below 2 dynes/cm\textsuperscript{2}. Rates of flow vary enormously within the circulation, being highest in arteries and arterioles (10 to 50 dynes/cm\textsuperscript{2}) (17) and lowest in the postcapillary venules (1 to 4 dynes/cm\textsuperscript{2}) (2, 12). Therefore, our data suggest that \textit{C. albicans} is likely to predominantly adhere to the endothelial lining of small capillaries and postcapillary venules. This is similar to the migration of leukocytes into tissues during inflammation, which also occurs predominantly in postcapillary venules (15).

In contrast to static assays, using conditions of flow revealed that yeast forms of \textit{C. albicans} adhere in greater numbers than do pseudohyphal and hyphal cells. One possible explanation for this phenomenon is that differences in the culture conditions used to generate the different morphological forms of \textit{Candida} may alter the gene expression of cell wall molecules that could be responsible for the differences in adhesion rather than the morphological form itself. To address this issue, we generated yeast and hyphal forms of CAF2-1 by using constant pH but varying the temperature and also cultured \textit{Candida} by using different carbon sources. In addition, we used a mutant strain of \textit{C. albicans} (SSY50-B) in which yeast-to-hyphal conversion can be tightly regulated (23). SSY50-B is a good control for the effect of growth conditions on adhesion, as both yeast forms and hyphae are cultured under identical conditions apart from the addition of doxycycline to induce hyphal transformation. Under all conditions tested, yeast forms adhered to endothelial monolayers in significantly greater numbers than did hyphal forms, suggesting that the increased binding of yeast forms is independent of culture conditions.

The preferential adhesion of CAF2-1 yeast forms suggests that the adhesion of SSY50-B yeast forms is unlikely to simply be a reflection of their genetic modification but more likely reflects true differences in adhesive properties between the different morphological forms. Our data showing that yeast forms adhere in greater numbers under conditions of flow are in contrast to evidence from our own, and previously published, static assays which show that germinated candidal forms exhibit greatest adhesion to endothelial cells (20). The difference in binding of the two forms of \textit{Candida} under static and flow conditions highlights the importance of replicating as closely as possible the nature of \textit{Candida}-endothelial interactions in vivo when studying adhesion to endothelium.

The suggestion that yeast forms rather than hyphal forms of \textit{Candida} preferentially bind to the endothelium may not be as controversial as first thought. Previous in vitro studies showed that yeast forms adhere to the endothelial cell surface before undergoing germination and causing endothelial damage (6, 19). In addition, other groups have reported that a mutant strain of \textit{C. albicans}, defective in filament formation, can leave the circulation in vivo (3, 4, 24). We have also previously shown that yeast forms of SSY50-B extravasate from blood vessels into tissues in vivo without undergoing morphogenic change (23). Furthermore, \textit{Candida glabrata} and \textit{Candida parapsilosis} also cause disseminated candidiasis despite their inability to form true hyphae (22). Taken together, these data suggest that yeast forms of \textit{C. albicans} can adhere to the endothelium. This has led some to suggest that yeast cells may be better adapted for free dissemination within the circulation because of their more compact size and shape (9).

We found that \textit{C. albicans} adhesion to the endothelium was extremely rapid and occurred with no evidence of rolling behavior like that characterizing selectin-mediated leukocyte binding (16). Similarly, Glee et al. noted that free-flowing \textit{C. albicans} cells in one frame were adherent in the next (8). This evidence suggests that \textit{Candida} binding to endothelium is rapid, occurring in less than 34 ms. This is supported by in vivo experiments that show \textit{C. albicans} clearance from the circulation within 15 minutes in animal models of systemic candidiasis (5, 18). Like Glee et al., we also noted that all three morphogenic forms of \textit{C. albicans} exhibited both homotypic adhesion to adherent \textit{Candida} cells and heterotypic adhesion to the endothelium (8). However, further investigations are needed to determine the binding efficiency and specificity of \textit{Candida} adhesins involved in these different types of adhesion.

The orientation of free-flowing pseudohyphal and hyphal forms, with the mother cell in front and the hyphal extension behind, likely represents the optimum hemodynamic configuration. Given this, it was surprising that hyphal and pseudohyphal forms appeared to adhere to endothelial cells by their hyphal tip rather than by the mother cell body. This finding suggests either that pseudohyphal and hyphal forms adhere to different endothelial ligands than yeast forms do or that the distribution of adhesion ligands changes on conversion from yeast to pseudohyphal or hyphal forms. It is known that newly synthesized cell wall and membrane-anchored proteins will be inserted at the hyphal tip; for example, Hwp1 (hyphal wall protein 1) expression is concentrated at the tip of hyphal cells (P. Sudbery, P. Amornrattanapan, and J. Berman, unpublished observation). Further studies to identify the specific adhesion ligands expressed by the different forms of \textit{C. albicans} and their respective receptors on endothelial cells are warranted.

In conclusion, we found that \textit{C. albicans} adhesion to endothelium under physiological conditions of flow was rapid and the adhesion of yeast forms was significantly greater than that of pseudohyphal forms, which in turn was significantly more than that of hyphal forms. Initially these results seem surprising because hyphae are generally thought to be more “sticky” than yeast. However, they are consistent with the general model of \textit{Candida} infection and with recent in vivo data sug-
gesting that yeast forms may be capable of adhering to the endothelium and migrating into the tissues before undergoing morphicogenic change to cause tissue damage. The challenge now is to identify the precise nature of the *C. albicans* adhesins and endothelial receptors responsible for adhesion of the different morphological forms of *C. albicans* to endothelial cells under physiological conditions of flow.

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